

CLAIMS

What is claimed is:

1. An RNA aptamer that selectively binds a coagulation pathway factor, the RNA aptamer having a dissociation constant for the coagulation pathway factor of about 20 nanomolar (nM) or less.

2. The RNA aptamer of claim 1, wherein the coagulation pathway factor is selected from the group consisting of prothrombin, thrombin, IX, IXa, X, Xa, VII, VIIa and combinations thereof.

3. The RNA aptamer of claim 2, wherein the factor is IXa, and the RNA aptamer further comprises a consensus sequence comprising AUA.

4. The RNA aptamer of claim 3, wherein the dissociation constant ranges from about 100 pM to about 10 nM.

5. The RNA aptamer of claim 4, wherein the dissociation constant ranges from about 400 pM to about 10 nM.

6. The RNA aptamer of claim 2, wherein the coagulation pathway factor is VIIa, and the dissociation constant ranges from about 100 pM to about 10 nM.

7. The RNA aptamer of claim 6, wherein the dissociation factor ranges from about 400 pM to about 10 nM.

8. The RNA aptamer of claim 2, wherein the coagulation pathway factor is Xa, and the dissociation constant ranges from about 100 pM to about 10 nM.

9. The RNA aptamer of claim 8, wherein the dissociation constant ranges from about 400 pM to about 10 nM.

10. The RNA aptamer of claim 2, wherein the coagulation pathway factor is thrombin, and the dissociation constant ranges from about 100 pM to about 10 nM.

11. The RNA aptamer of claim 10, wherein the dissociation constant ranges from about 400 pM to about 10 nM.

12. The RNA aptamer of claim 1, further comprising at least one modified nucleotide.

13. An RNA aptamer comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:1-47, or a truncate thereof.

14. The RNA aptamer of claim 13, wherein the truncate is SEQ ID

NO:70 or SEQ ID NO:71.

15. An RNA aptamer that selectively binds thrombin, the aptamer comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:50-56, and SEQ ID NOs:48 and 49 flanking the nucleotide sequence on the 5' and 3' ends, respectively; or a truncate thereof.

16. The RNA aptamer of claim 15, wherein the truncate is SEQ ID NO:57.

17. An RNA aptamer that selectively binds thrombin, the aptamer comprising the sequence AACAA.

18. An RNA aptamer that selectively binds factor VIIa, the aptamer comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:74-101, and SEQ ID NOs:102 and 103 flanking the nucleotide sequence on the 5' and 3' ends, respectively; or a truncate thereof.

19. An RNA aptamer that selectively binds factor Xa, the aptamer comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:104-147, and SEQ ID NOs:102 and 103 flanking the nucleotide sequence on the 5' and 3' ends, respectively; or a truncate thereof.

20. A composition comprising a therapeutically effective amount of an RNA aptamer of claim 1, and a pharmaceutically acceptable diluent or vehicle.

21. A method of modulating the biological activity of a coagulation pathway factor, the method comprising:

- (a) administering to a warm blooded vertebrate in need thereof an effective amount of an RNA aptamer of claim 1; and
- (b) modulating the biological activity of the coagulation pathway factor in the warm-blooded vertebrate through the administering of the RNA aptamer in step (a).

22. The method of claim 21, wherein the administering is selected from the group consisting of intravenous administration, intrasynovial administration, transdermal administration, intramuscular administration, subcutaneous administration and topical administration to a blood vessel.

23. The method of claim 21, wherein the vertebrate is a mammal.

24. A method of treating cardiovascular disease in a warm blooded vertebrate, the method comprising administering an effective amount of an

RNA aptamer of claim 1 to a vertebrate subject suffering from cardiovascular disease, whereby cardiovascular disease in the vertebrate subject is treated.

25. The method of claim 24, wherein the administering is selected from the group consisting of intravenous administration, intrasynovial administration, transdermal administration, intramuscular administration, subcutaneous administration and topical administration to a blood vessel.

26. The method of claim 24, wherein the vertebrate is a mammal.

27. An RNA aptamer that selectively binds an E2F family member, the RNA aptamer having a dissociation constant for the E2F family member of about 20 nM or less.

28. The RNA aptamer of claim 27, wherein the dissociation constant ranges from about 100 pM to about 10 nM.

29. The RNA aptamer of claim 28, wherein the dissociation constant ranges from about 400 pM to about 10 nM.

30. The RNA aptamer of claim 27, further comprising at least one modified nucleotide.

31. An RNA aptamer that selectively binds an E2F family member, the aptamer comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:64-69 and 189-228, wherein SEQ ID NOs:62 or 188 flank the nucleotide sequence on the 5' end and SEQ ID NO:63 flanks the nucleotide sequence on the 3' end; or a truncate thereof.

32. A composition comprising a therapeutically effective amount of an RNA aptamer of claim 27, and a pharmaceutically acceptable diluent or vehicle.

33. A method of modulating E2F activity in a warm-blooded vertebrate in which said modulation is desired, the method comprising:

- (a) administering to the warm-blooded vertebrate an effective amount of an RNA aptamer of claim 27; and
- (b) modulating E2F activity in the warm-blooded vertebrate through the administering of the RNA aptamer of step (a).

34. The method of claim 33, wherein the administering is selected from the group consisting of intravenous administration, intrasynovial administration, transdermal administration, intramuscular administration, subcutaneous administration and topical administration to a blood vessel.

35. The method of claim 33, wherein the vertebrate is a mammal.

36. A method of identifying a ligand to a target from a candidate mixture of potential ligands, the method comprising:

- (a) preparing a candidate mixture of potential ligands;
- 5 (b) contacting the candidate mixture with the target in a low stringency buffer, wherein ligands having increased affinity to the target relative to the candidate mixture bind to the target;
- (c) removing unbound candidate mixture;
- 10 (d) collecting the ligands that are bound to the target to produce a first collected ligand mixture;
- (e) contacting the first collected ligand mixture with the target in a higher stringency buffer, wherein ligands having increased affinity to the target relative to the first collected ligand mixture bind to the target;
- 15 (f) removing unbound first collected ligand mixture; and
- (g) collecting the ligands that are bound to the target to for a second collected ligand mixture thereby identify ligands to the target.

37. The method of claim 36, further comprising step (i): repeating steps (e), (f) and (g) one or more times with a higher stringency buffer each time.

38. The method of claim 36, further comprising amplifying the first collected ligand mixture or the second collected ligand mixture, or both, to yield a ligand enriched mixture, whereby a ligand to the target is identified.

39. The method of claim 36, wherein the ligand mixture comprises a candidate mixture of nucleic acids.

40. The method of claim 36, wherein the candidate mixture of nucleic acids comprises single strand nucleic acids.

41. The method of claim 40, wherein the single stranded nucleic acids are ribonucleic acids.

30 42. The method of claim 40, wherein the single stranded nucleic acids are deoxyribonucleic acids.

43. The method of claim 41, wherein said candidate mixture of nucleic acids comprises 2'-modified ribonucleic acids.

44. The method of claim 43, wherein said 2'-modified ribonucleic

acids comprise 2'-fluoro (2'-F) modified nucleic acids.

45. The method of claim 36, wherein the more stringent buffer comprises a physiological buffer.

46. A product identified by the process of claim 36.

5 47. A method of identifying a ligand selective for a target from a candidate mixture of potential ligands for the target, the method comprising:

- (a) providing a target selected from a first species of organism;
- (b) preparing a candidate mixture of potential ligands;
- (c) contacting the candidate mixture with the target, wherein ligands
10 having increased affinity to the target from the first species relative to the candidate mixture bind to the target from the first species;
- (d) removing unbound candidate mixture;
- (e) collecting the ligands that are bound to the target from the first
15 species to produce a first collected ligand mixture;
- (f) contacting the first collected ligand mixture with a target from a second species of organism, the target from the second species having at least a portion thereof that is substantially homologous to the same portion in the target from the first species, wherein
20 ligands having increased affinity to the target from the second species relative to the first collected ligand mixture bind to the target;
- (g) removing unbound first collected ligand mixture; and
- (h) collecting the ligands that are bound to the target from the
25 second species to form a second collected ligand mixture to thereby identify ligands to the target.

48. The method of claim 47, further comprising step (i): repeating steps (f), (g) and (h) one or more times, wherein each additional time alternates between the target from the first species and the target from the
30 second species.

49. The method of claim 47, further comprising amplifying the first collected ligand mixture, the second collected ligand mixture, or both, to yield a ligand enriched mixture, whereby a ligand to the target is identified.

50. The method of claim 47, wherein the ligand mixture comprises a

candidate mixture of nucleic acids.

51. The method of claim 50, wherein the candidate mixture of nucleic acids comprises single strand nucleic acids.

52. The method of claim 51, wherein the single stranded nucleic acids are ribonucleic acids.

53. The method of claim 51, wherein the single stranded nucleic acids are deoxyribonucleic acids.

54. The method of claim 52, wherein said candidate mixture of nucleic acids comprises 2'-modified ribonucleic acids.

55. The method of claim 54, wherein said 2'-modified ribonucleic acids comprise 2'-fluoro (2'-F) modified nucleic acids.

56. A product identified by the process of claim 47.

57. An RNA aptamer that selectively binds Ang1 or Ang2, the RNA aptamer having a dissociation constant for Ang1 or Ang2 of about 20 nM or less.

58. The RNA aptamer of claim 57, wherein the dissociation constant ranges from about 100 pm to about 10 nM.

59. The RNA aptamer of claim 58, wherein the dissociation constant ranges from about 400 pm to about 10 nM.

60. The RNA aptamer of claim 57, further comprising at least one modified nucleotide.

61. An RNA aptamer that selectively binds Ang1, the aptamer comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:151-165, and SEQ ID NOs:149 and 150 flanking the nucleotide sequence on the 5' and 3' ends, respectively; or a truncate thereof.

62. An RNA aptamer that selectively binds Ang2, the aptamer comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:168-186, and SEQ ID NOs:166 and 167 flanking the nucleotide sequence on the 5' and 3' ends, respectively; or a truncate thereof.

63. The RNA aptamer of claim 62, wherein the truncate is SEQ ID NO:187.

64. A composition comprising a therapeutically effective amount of an RNA aptamer of claim 57, and a pharmaceutically acceptable diluent or vehicle.

65. A method of modulating Ang1 or Ang2 activity in a warm-blooded vertebrate in which said modulation is desired, the method comprising:

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- (a) administering to the warm-blooded vertebrate an effective amount of an RNA aptamer of claim 57; and
 - (b) modulating Ang1 or Ang2 in the warm-blooded vertebrate through the administering of the RNA aptamer of step (a).

66. The method of claim 65, wherein the administering is selected from the group consisting of intravenous administration, intrasynovial
10 administration, transdermal administration, intramuscular administration, subcutaneous administration and topical administration to a blood vessel.

67. The method of claim 65, wherein the vertebrate is a mammal.

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